Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

UV-B radiation reduces *in vitro* germination of *Metarhizium anisopliae* s.l. but does not affect virulence in fungus-treated *Aedes aegypti* adults and development on dead mosquitoes

M.L. Falvo[1,2], R.A. Pereira-Junior[1], J. Rodrigues[1], C.C. López Lastra[2], J.J. García[2], É.K.K. Fernandes[1] and C. Luz[1]

1 Instituto de Patologia Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás, Goiânia (UFG), GO, Brasil
2 Centro de Estudios Parasitológicos y de Vectores (CEPAVE), Universidad Nacional de La Plata-CONICET, La Plata, Buenos Aires, Argentina

Keywords
application, biological control, entomopathogenic fungus, mosquito, UV-B tolerance.

Correspondence
Christian Luz, Instituto de Patologia Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás (UFG), Avenida Esperança s/n, Campus Samambaia, 74690-900 Goiânia, GO, Brazil.
E-mail: wchrisluz@hotmail.com

2016/1316: received 16 June 2016, revised 2 September 2016 and accepted 25 September 2016

doi:10.1111/jam.13309

Abstract

Aims: Control of diurnal *Aedes aegypti* with mycoinsecticides should consider the exposure of fungus-treated adults to sunlight, and especially to UV-B radiation that might affect activity of conidia applied on the mosquito's surface.

Methods and Results: Germination of *Metarhizium anisopliae* s.l. IP 46 conidia on SDAY medium was not affected at the lowest level of radiation with UV-B, 0·69 kJ m^{-2}, but was retarded and reduced at higher 2·075 and 4·15 kJ m^{-2}, and completely inhibited at ≥8·3 kJ m^{-2}. In contrast, germination of conidia applied onto fibreglass nettings and exposed from 0 to 16·6 kJ m^{-2} did not differ significantly among levels of irradiance exposure and the controls. There was also no significant impact of UV-B up to 16·6 kJ m^{-2} on the adulticidal activity of IP 46 and on the subsequent conidiogenesis on cadavers. The Quaite-weighted UV-B irradiance in the laboratory (1152 mW m^{-2}) was higher than the natural sunlight irradiance observed in the city of Goiânia in Central Brazil on midday (706 mW m^{-2} in August to 911 mW m^{-2} in October 2015).

Conclusions: UV-B does not impair the activity of IP 46 conidia applied previously to radiation on *A. aegypti* adults.

Significance and Impact of the Study: Findings contribute to a better understanding of the effectiveness of *M. anisopliae* against day-active *A. aegypti* and its potential for biological mosquito control.

Introduction

Aedes aegypti lives in close contact with humans in urban areas in the subtropics and tropics (Harrington *et al.* 2001). Adults are active during the day and eventually transmit viral infections such as dengue, chikungunya and Zika fever (Vega-Rúa *et al.* 2015; Carneiro and Travassos 2016; Marcondes and Ximenes 2016). The circadian rhythm of these vectors includes two maximum peaks of flight activity, one early in the morning and another 2 h before sunset (Taylor and Jones 1969). During these periods, adults are foraging for nectar and blood sources, swarming, mating and seeking oviposition and resting sites (Trpis *et al.* 1973; Chadee and Martinez 2000; Cabrera and Jaffe 2007; Wong *et al.* 2011).

Entomopathogenic fungi that infect and kill different developmental stages of the mosquito gained interest as a biological control. *Metarhizium anisopliae* is among the most prominent candidates as this species is highly active against *A. aegypti* eggs, larvae and especially adults (Scholte *et al.* 2004, 2007; Luz *et al.* 2007; Albernaz *et al.* 2009; Leles *et al.* 2010). Infectious conidia that contact and adhere to the insect cuticle may eventually germinate and invade their host through the cuticle causing an infection that results in the death of the host. Recent efforts to develop mosquito control with mycoinsecticides

focus on trap technology where target adult vectors are contaminated and infected, and pathogens are spread among the host population after leaving the trap (Snetselaar *et al.* 2014).

Environmental exposure to ultraviolet radiation represents a major drawback for the fungal activity in diurnal insects that are temporarily exposed to sunlight. The viability of *M. anisopliae* conidia and their virulence against target insects were clearly affected by sunlight (Braga *et al.* 2001a, 2015), and UV-B radiation (315–280 nm) has been shown to be the most harmful to *M. anisopliae* and other entomopathogens (Fernandes *et al.* 2015). *In vitro* germination of *M. anisopliae* conidia and the development of colonies on a culture medium were delayed or inhibited in direct proportion to the dose of UV-B (Braga *et al.* 2001b).

Aedes aegypti adults were highly susceptible to *M. anisopliae* under laboratory conditions (Scholte *et al.* 2007; Leles *et al.* 2010) without exposure to UV radiation, but there is no information about the effectiveness of this fungus against adults treated with conidia and then promptly exposed to UV-B radiation. We report here *in vitro* and *in vivo* tests about the effect of UV-B on the germination and virulence of *M. anisopliae* s.l. against *A. aegypti* adults respectively.

Materials and methods

Origin and preparation of the fungus

Metarhizium anisopliae s.l. IP 46 was isolated in 2001 from a soil sample collected in the State of Goiás, in Central Brazil (Rocha *et al.* 2013). The fungus was cultured on Potato Dextrose Agar medium (Himedia® Laboratories. Pvt. Ltd., India) in Petri dishes (90 mm diameter × 10 mm height) and maintained for 15 days at 27 ± 1°C and 12 h photophase. Conidia were scraped with a spatula from the surface of the culture, dried in a desiccator with silica gel for 48 h at 4 ± 1°C and immediately used. Approximately 0·01 g of conidia were suspended in 50 ml 0·01% polyoxyethylene sorbitan monooleate (Tween 80®; Sigma Chemical Co., São Paulo, SP, Brazil) vortexed, and the suspension filtered through hydrophilic cotton; the number of conidia/weight was determined with a haemocytometer. Conidial viability (>95%) was assessed by inoculating 20 μl of 10^6 conidia ml^{-1} suspension in a Petri dish with Sabouraud dextrose agar and yeast extract (quarter strength SDAY: peptone 2·5 g l^{-1}, dextrose 10 g l^{-1}, yeast extract 2·5 g l^{-1}, agar 10 g l^{-1}) medium amended with benomyl (0·002% w/v, 50% active ingredient; Benlate®, DuPont, São Paulo, SP, Brazil) and chloramphenicol (0·05% w/v). The percentage of germinated conidia was determined after 24 h of incubation at 25 ± 1°C and 12 h photophase. Conidia were considered germinated when the elongating germ tube was longer than the maximum conidial diameter (Fernandes *et al.* 2007).

Origin, rearing and preparation of mosquitoes

Aedes aegypti originated from larvae collected in 2012 in Goiânia, Brazil, and the subsequent colony was reared under laboratory conditions at 27 ± 5°C, 75 ± 10% relative humidity (RH) and natural photophase. Adults were maintained in a cage (50 cm height × 50 cm width × 40 cm depth) covered on all sides with a nylon mesh. They were continuously offered a 10% saccharose solution on piece of filter paper placed upright in a small glass container. In addition, females were allowed to feed twice a week on mice as described by Lima *et al.* (2009), a technique approved previously by the Ethics Commission for the Use of Animals of the Federal University of Goiás, Goiânia (CEUA 079/13, UFG, February 10, 2014). At the bottom of the cage, a filter paper (15 cm length × 7 cm width) dipped partially in an amber glass cup with water, was arranged in order to permit females to oviposit. The filter papers with eggs were removed daily, arranged in another cup as mentioned, and incubated for 48 h to allow the completion of embryogenesis. Eggs on filter paper with fully developed but uneclosed larvae were then dried and stored in plastic bags at ambient temperature.

Larvae eclosed when filter papers with embryonated eggs were transferred into a plastic bowl with 2000 ml of tap water. Small amounts of ground cat food pellets (Black Jack; Alisul Alimentos S.A., São Leopoldo, Rio Grande do Sul, Brazil) were dusted on alternate days onto the water surface to feed larvae. Pupae were transferred routinely to a screened plastic cup (7 cm diameter × 10 cm height) with 35 ml tap water, and emergent adults were transferred into the mentioned cage.

To begin the experiments, adults were aged to 48–120 h postemergence at 25 ± 1°C and 75 ± 10% RH and were fed saccharose solution as mentioned previously.

UV-B radiation

In the laboratory, UV-B radiation was provided with four ultraviolet lamps (UVB-313 EL; Q-Lab Corporation, Cleveland, OH) in a chamber (133 cm width × 86 cm height × 50 cm depth) made of wood precisely for UV-B radiation purpose. Plastic dishes containing either mosquitoes (90 mm diameter × 10 mm height), a piece of net or conidia on solid culture media (60 mm diameter × 10 mm height), were covered with a transparent

polyethylene film (i.e. food wrap) (3M®, Manaus, Brazil) that retained adults in the dishes and had no effect on the passage of the UV-B. Dishes were placed at a 30 cm distance from the UV-B lamps and covered with a 0·13 mm-thick cellulose diacetate film (JCS Industries, Le Mirada, CA), which blocked radiation below 290 nm, including UV-C radiation (radiation of 100−280 nm is not transmitted to the biosphere). The spectral distribution of the filtered lamps of the chamber is given in Fig. 1. Spectral irradiance was measured with an Ocean Optics Spectroradiometer (Ocean Optics, USB2000 + RAD, Dunedin, FL). Dishes were exposed to 1152 mW m^{-2} of Quaite-weighted UV-B irradiance (Quaite *et al.* 1992) for 0 (wrapped with aluminium foil), 10, 30, 60, 120, 180 and 240 min resulting to final doses of 0 (control), 0·69, 2·075, 4·15, 8·3, 12·45 and 16·6 kJ m^{-2} respectively. The temperature during the exposure was maintained at 28 ± 2°C. Additionally, natural UV-B radiation was measured every month between August and November 2015 with an Ocean Optics Spectroradiometer at 10 a.m., noon and 3 p.m. in the city of Goiânia, Brazil, during cloudless, sunny days. These measurements were recorded for comparison between the intensity of natural UV-B of local sunlight and the radiation obtained artificially in the chamber in the laboratory.

In vitro assays

Pieces of inorganic large meshed fibreglass nettings (1 cm^2 surface with a total 36 holes cm^{-2}) were sterilized and treated with the fungus. Dry conidia were spread uniformly with a sterilized brush (Pinctore Tigre®, 8158, Tigre S. A., Castro, PR, Brazil) to a final concentration of approximately 10^6 conidia cm^{-2}. Each treated piece of netting was set in the bottom of a dish (60 mm diameter × 10 mm height) with no lid, covered with a polyethylene film and the cellulose diacetate film, and exposed to UV-B. One of the dishes containing a treated net was covered with aluminium foil as a control and left in the UV-B chamber for 4 h. For conidial viability tests, each piece of net was suspended after exposure in 1 ml of aqueous Tween 80®, 0·01% v/v, vortexed for 2 min, and the resulting conidial suspensions filtered. Petri dishes with SDAY medium with benomyl (0·002% w/v) and chloramphenicol (0·05% w/v) were inoculated with 20 μl of the conidial suspension. Plates were incubated for 24, 48 and 72 h at 25 ± 1°C in the dark, and then treated with a thin layer of Amann lactophenol and cotton blue solution. The relative germination was determined by observing a total of 400 conidia; conidia were scored as germinated if the elongating germ tube was longer than the maximum conidial diameter.

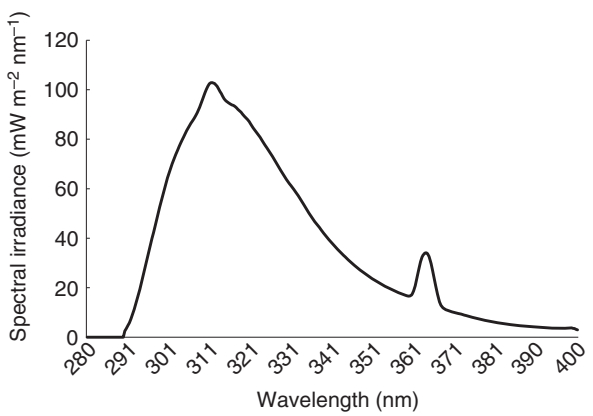

Figure 1 Spectral irradiance of four lamps providing radiation at a total of 1152 mW m^{-2} of Quaite-weighted UV-B irradiance (Quaite *et al.* 1992).

In another assay, 20 μl of a conidial aqueous 0·01% Tween 80 suspension, at a concentration of 10^6 conidia ml^{-1}, were inoculated on SDAY medium amended with benomyl (0·002% w/v) and chloramphenicol (0·05% w/v) in the centre of a Petri dish (60 mm diameter × 10 mm height). After evaporation of the liquid, dishes were covered with the polyethylene and the cellulose diacetate films; conidia were then irradiated, and germination determined as mentioned.

In vivo assays

The inner surface of plastic containers provided with a lid (7 cm diameter × 10 cm height, 333 cm^{-2} total surface) was uniformly roughened with a sandpaper (Carborundum®, carbomassa A150, no12E, Vinhedo, SP, Brazil), set about 50 orifices of 2–3 mm diameter evenly in the lid and upper part of the container, rinsed with water, dried and sterilized by direct exposure to ultraviolet radiation (UV-C Lamp Germicidal Ultraviolet G30T8; Royal Philips Electronics, Amsterdam, the Netherlands) for 20 min. Dry conidia were applied on the whole inner surface of the container with a previously sterilized brush (Pinctore Tigre®, 8158) at 3·3 × 10^5 conidia cm^{-2}. Adults were maintained for 6 h in the fungus-treated container or in an untreated (control) container at 25 ± 1°C and 75 ± 5% RH. During this period (between 7 a.m. and 1 p.m.) insects were active. Containers were arranged in a plastic chamber with a saturated solution of NaCl in order to maintain humidity at 75% (Winston and Bates 1960).

Mosquitoes were then immobilized with CO_2 for 15 s, and three males and females were transferred to the bottom of a plastic dish (90 mm diameter × 10 mm height) covered with a polyethylene film to keep mosquitoes in the dish. Then dishes with adults treated with conidia or not (control) were covered with the cellulose diacetate

film, and exposed to UV-B with the same procedure and doses of radiation as mentioned. Subsequently, a total of 12 adults for each exposure time were transferred to a plastic container (7 cm diameter × 10 cm height) with sugar solution as described above and covered with gauze.

Containers were incubated in a plastic chamber for 15 days at 25 ± 1°C, 75 ± 5% RH and 12 h photophase. Mortality was monitored and recorded daily; dead individuals were removed to water agar (1% w/v) amended with chloramphenicol (0·05% w/v), thiabendazole (0·0004% w/v) and crystal violet (0·001% w/v) in a Petri dish (60 mm diameter × 10 mm height), and incubated at 25 ± 1°C for 10 days to allow the development of conidia on infected adults. Then mosquitoes were gently removed, suspended in a tube with 5 ml 0·01% Tween 80, the suspension vortexed for 2 min, filtered as mentioned, and finally the number of conidia per cadaver was quantified with a haemocytometer.

Data analysis

All tests were carried out with four independent repetitions each. Percentage values of germination and adult mortality were arcsine-square root transformed. These and the numbers of conidia on dead adults were analysed with ANOVA and the Student–Newman–Keuls multiple range test for comparison of means. Means were considered to be significantly different at $P < 0·05$. Lethal times to kill 50 and 90% of adults (LT_{50} and LT_{90}) and their respective 95% confidence intervals (C.I.) were calculated by probit analysis for dependent values (Throne *et al.* 1995).

Results

Germination *in vitro*

Relative germination of conidia exposed on SDAY to UV-B radiation began in the first 24 h after exposure to doses ≤4·15 kJ m^{-2}. Cumulative germination increased at these lower doses and reached the highest values (≥99%) in the control (not exposed to UV-B), and was lowest at 0·69 kJ m^{-2}, 72 h after exposure. After irradiation with 8·3 kJ m^{-2}, germination reached 5·4 ± 2·5% at 72 h, and no conidia germinated when exposed to higher doses tested at this moment (≥12·45 kJ m^{-2}; Fig. 2a). There was a high significant effect of the UV-B dose on germination up to 72 h of exposure on SDAY medium ($F_{6,21} \geq 14·1$; $P < 0·001$) with reduced germination of conidia exposed to higher doses.

For conidia applied to netting and exposed to UV-B radiation, germination varied from a maximum of 48 ± 4% (control with no exposure to UV-B) to a minimum of 1·5 ± 0·5% (16·6 kJ m^{-2}) after 24-h incubation, with a highly significant effect of the dose on the germination ($F_{6,21} = 9·9$; $P < 0·001$; $0 > 4·15 > 16·6$ kJ m^{-2}), causing a reduced germination of conidia exposed to higher doses. Germination increased in the following hours and reached maxima of 65·7 ± 2·2% (control) and 36·1 ± 3·2% (16·6 kJ m^{-2}) at 72 h of incubation without a significant effect of the dose on germination ($F_{6,21} = 2·5$; $P = 0·06$; Fig. 2b).

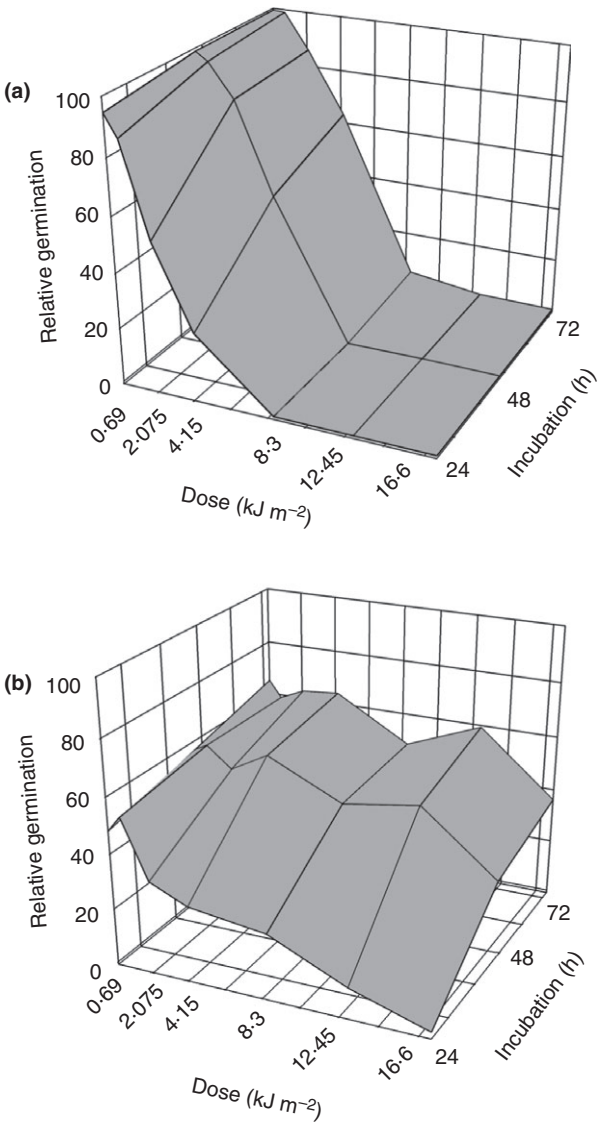

Figure 2 Cumulative germination of *Metarhizium anisopliae* IP 46 conidia applied on SDAY supplemented with chloramphenicol (0·05% w/v) and benomyl (0·002% w/v) (a) or meshed net (b), exposed to UV-B (at 0 up to 16·6 kJ m^{-2}) and subsequently incubated on SDAY added with the same antibiotic and fungicide up to 72 h at 25 ± 1°C.

Mortality of adults

The first adults treated with conidia and immediately exposed to UV-B died within 24 h (2·1 ± 1% at 4·15 kJ m^{-2}). Cumulative mortality increased in the following days, regardless of the tested dose and, after 15 days, varied from 70·8 ± 5·2% (0 kJ m^{-2}) to 43·8 ± 10·4% (12·45 kJ m^{-2}) without a significant effect of the dose on mortality ($F_{6,21}$ = 0·51; P = 0·79; Fig. 3). Lethal times to kill 50% and 90% of fungus-treated adults varied from 9·7 days (control individuals not exposed to UV-B) to 18 days (2·075 kJ m^{-2}), and 21·3 days (0 kJ m^{-2}) to 37·5 days (2·075 kJ m^{-2}), respectively, without significant difference among values for both LT_{50} and LT_{90} based on their confidence intervals (Table 1).

Development of conidia on dead adults

Mycelium was detected on the cuticle within 24 h of incubation of cadavers on water agar, and conidiogenesis of IP 46 started within 2–3 days regardless of the dose tested. The mean number of conidia per cadaver after a 10-day incubation was highest at 1·13 × 10^7 ± 1·5 × 10^6 (at 12·45 kJ m^{-2}) and lowest for the control cadavers (7·1 × 10^6 ± 4·5 × 10^5) without previous exposure of fungus-treated adults to UV-B. There was no significant effect of the dose on the number of conidia ($F_{6,21}$ = 0·38; P = 0·89; Fig. 4).

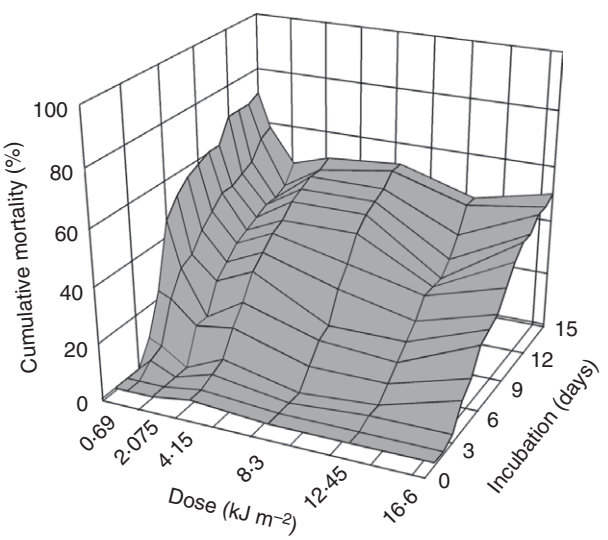

Figure 3 Cumulative mortality of *Aedes aegypti* adults after treatment with *Metarhizium anisopliae* IP 46 conidia, exposure to UV-B (up to 16·6 kJ m^{-2}) and incubation at 25 ± 1°C, 75% relative humidity and 12 h photophase up to 15 days.

Table 1 Lethal time (days) to kill 50% or 90% (LT_{50} and LT_{90}) with their respective confidence interval and slope ± standard error of the mean of *Aedes aegypti* adults after treatment with *Metarhizium anisopliae* s.l. conidia and exposure to different doses of UV-B and subsequent incubation at 25 ± 1°C and 75 ± 10% relative humidity

	Lethal time (C.I.)		
Dose (kJ m^{-2})	50%	90%	Slope ± SE
0	9·7 (2·4–27·9)	21·3 (13–123·6)	0·11 ± 0·02
0·69	14·2 (6·2–33·6)	29·1 (19·1–97·6)	0·08 ± 0·02
2·075	18 (12·8–28·6)	37·5 (27·4–68·8)	0·07 ± 0·02
4·15	16·6 (8·6–41·2)	34·3 (22·3–110)	0·07 ± 0·02
8·3	14·2 (9·7–24)	25·6 (18·7–54·9)	0·11 ± 0·02
12·45	16·8 (13·3–23·2)	29·7 (23·3–45·3)	0·10 ± 0·02
16·6	16·7 (8·7–41·3)	34·2 (22·2–109·6)	0·07 ± 0·02

Values, based on four repetitions at 3·3 × 10^5 conidia cm^{-2}; cumulative control mortality (no exposure to conidia and UV-B) was 16·7 ± 5·1% and 29·2 ± 2·7% after exposure to UV-B without conidia at a 15-day incubation respectively.

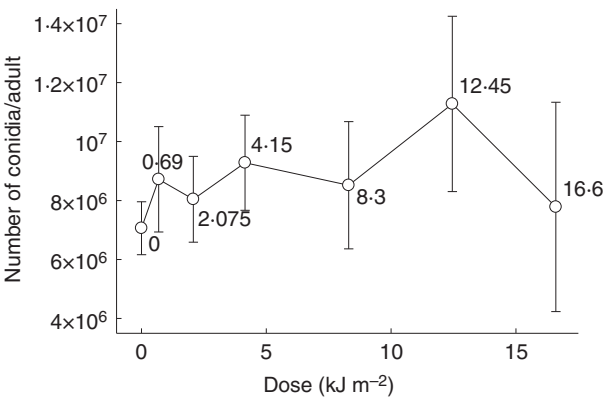

Figure 4 Mean number (± standard error of the mean) of *Metarhizium anisopliae* IP 46 conidia per dead *Aedes aegypti* adult, treated previously with the fungus and exposed to UV-B (up to 16·6 kJ m^{-2}). Cadavers were incubated in a humid chamber at 25 ± 1°C for 10 days.

Natural UV-B radiation in urban area in Central Brazil

The measured hourly ambient doses of UV-B in the city of Goiânia, in Central Brazil, between 10 a.m. and 3 p.m. increased slightly between August and November 2015. It was generally highest at midday (2·55 kJ m^{-2} h^{-1} in August–3·28 kJ m^{-2} h^{-1} in October) and lowest in the afternoon at 15 h (0·92–1·5 kJ m^{-2} h^{-1} in November; Fig. 5).

Discussion

Germination is the first step in fungal development after conidial adhesion to the insect cuticle, and is the essential process before the invasion of a susceptible insect host.

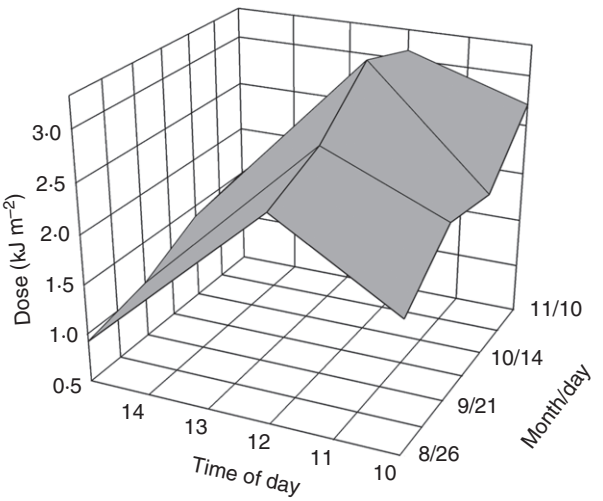

Figure 5 UV-B dose (kJ m^{-2}) h^{-1} registered between August and November 2015 in the city of Goiânia, Brazil, at 10 a.m., noon and 3 p.m. in the afternoon.

The progress of extra-cuticular development is prompted by nutrients but also depends on abiotic factors such as temperature and humidity. Sunlight, especially UV-B, affects the survival of conidia in the off-host environment (Fargues *et al.* 1996; Braga *et al.* 2001a,b; Rangel *et al.* 2004; Fernandes *et al.* 2007) but eventually also hampers spore viability on the surface of day-active target pest insects. The biocidal activity of UV-B against *M. anisopliae* conidia is well established under laboratory conditions (Braga *et al.* 2001a,b). Results in the present study emphasized the importance of the substrate to which conidia are applied while irradiated with UV-B. According to the substrate, conidia may have low- or high water availability, and vary regarding their susceptibility to UV-B (Fernandes *et al.* 2015). In fact, air-dried conidia of *Beauveria bassiana* s.l. were more tolerant to UV-B than fresh conidia (Le Grand and Cliquet 2013). In the current study, germination of conidia on SDAY was completely inhibited at doses ≥8·3 kJ m^{-2}. There was a clear harmful effect at lower doses that resulted in a slower and lower germination during the time of observation than in the controls. Germination was lowest after 0·69 kJ m^{-2} exposures. Exposures of conidia in a liquid film on the culture medium at 16·6 kJ m^{-2} eventually initiated metabolic activities in the conidia. Conidia exposed to ≥8·3 kJ m^{-2} did not show any distinct signs of germination and were clearly more susceptible to UV-B than were conidia applied onto nettings without any water film or nutrients, or onto the host's cuticle. Here, germination processes eventually were triggered but probably less advanced during any specific period of time than on the culture medium after the same exposure times.

Fortunately, adulticidal activity of IP 46 was not hampered under test conditions. A part of the conidia on the nettings or on the adults cuticle were probably not directly exposed to UV-B radiation, regardless of the tested dose, possibly due to the overlap of conidial layers or because conidia were covered by nylon (when on nets) or by cuticular structures (when adhered on adult mosquitoes). Consequently, a superior number of viable conidia maintained activity on the adults.

There was no clear relationship between the increase in susceptibility of conidia to UV-B doses tested either after application to the nettings or to the adulticidal activity. After contact with a fungus-treated substrate, conidia were found to be most prominently deposited on the ventral surfaces of the head, thorax and abdomen of adults than on the dorsal (upper) surfaces of the body. Possibly, mechanisms of protection and reactivation of conidia by visible light before or after exposure of fungus-treated adults to UV-B radiation sustained adulticidal activity (Braga *et al.* 2002; Brancini *et al.* 2016).

UV-B radiation did not clearly affect the rate of conidiogenesis on mosquito cadavers, and this could favour a propagation of the fungus among local populations of *A. aegypti*. However, there was an increasing variability of the number of conidia produced on dead adults at higher doses of UV-B radiation during initial infection. This means that the impact of microstructural patterns on the cuticle where conidia eventually adhered to—protected or not from radiation—on the further development and final conidiogenesis after host death might become more important at higher doses of UV-B radiation. At this moment, it is not known if initial quantitative inoculation of IP 46 conidia in *A. aegypti* adults influences conidiogenesis on cadavers as shown for *Beauveria bassiana* isolates on *Triatoma infestans* (Luz *et al.* 1999).

Results emphasized the importance of an indirect application of conidia as the best option for inoculating day-active mosquitoes such as *A. aegypti*. The highest UV-B doses (16·6 kJ m^{-2}) tested in laboratory conditions were distinctly higher than those measured under field conditions between August and November on cloudless days in the tropical Brazilian sites where *A. aegypti* is common. Results suggested that UV-B has no decisive negative impact on adulticidal activity of *M. anisopliae* in *A. aegypti* after indirect treatment with conidia. In addition, it is not expected that adults will remain exposed to sunlight for prolonged periods during their diurnal activities.

Fungus-treated netting or other substrates on which conidia can be deployed should be protected against both direct and (to the maximum reasonable extent) indirect sunlight in order to maintain viability, virulence and long-term persistence of conidia.

Acknowledgements

Authors thank Richard A. Humber (United States Department of Agriculture, Agricultural Research Service; Ithaca, NY) for the critical review of the manuscript. This research was funded by the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil) and Ministry of Education of Argentina (PPCP 016/2011), and by the Foundation for Research of the State of Goiás (FAPEG, Brazil, 201210267001060). We also thank CNPq for the grants: PQ 308850/2015-7 to É.K.K. Fernandes, and PQ 308189/2013-2 to C. Luz and ANPCyT (PICT 2012-0622) for a doctoral grant to M.L. Falvo.

Conflict of Interest

There are no conflicts of interest to declare.

References

Albernaz, D.A.S., Tai, M.H.H. and Luz, C. (2009) Enhanced ovicidal activity of an oil formulation of the fungus *Metarhizium anisopliae* on the mosquito *Aedes aegypti*. *Med Vet Entomol* **23**, 141–147.

Braga, G.U.L., Flint, S.D., Miller, C.D., Anderson, A.J. and Roberts, D.W. (2001a) Both solar UVA and UVB radiation impair conidial culturability and delay germination in the entomopathogenic fungus *Metarhizium anisopliae*. *Photochem Photobiol* **74**, 734–739.

Braga, G.U.L., Flint, S.D., Messias, C.L., Anderson, A.J. and Roberts, D.W. (2001b) Effect of UVB on conidia and germlings of the entmopathogenic hyphomycete *Metarhizium anisopliae*. *Mycol Res* **105**, 874–882.

Braga, G.U.L., Flint, S.D., Miller, C.D., Anderson, A.J. and Roberts, D.W. (2002) Damage and recovery from UV-B exposure in conidia of the entomopathogens *Verticillium lecanii* and *Aphanocladium album*. *Mycologia* **94**, 912–920.

Braga, G.U.L., Rangel, D.E.N., Fernandes, E.K.K., Flint, S.D. and Roberts, D.W. (2015) Molecular and physiological effects of environmental UV radiation on fungal conidia. *Curr Genet* **61**, 405–425.

Brancini, G.T.P., Rangel, D.E.N. and Braga, G.U.L. (2016) Exposure of *Metarhizium acridum* mycelium to light induces tolerance to UV-B radiation. *FEMS Microbiol Lett* **363**, 6, fnw036.

Cabrera, M. and Jaffe, K. (2007) An aggregation pheromone modulates lekking behavior in the vector mosquito *Aedes aegypti* (Diptera: Culicidae). *J Am Mosq Control Assoc* **23**, 1–10.

Carneiro, L.A.M. and Travassos, L.H. (2016) Autophagy and viral diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *Microbes Infect* **18**, 169–171.

Chadee, D.D. and Martinez, R. (2000) Landing periodicity of *Aedes aegypti* with implications for dengue transmission in Trinidad, West Indies. *J Vect Ecol* **25**, 158–163.

Fargues, J., Goettel, M.S., Smits, N., Ouedraogo, A., Vidal, C., Lacey, L.A., Lomer, C.J. and Rougier, M. (1996) Variability in susceptibility to simulated sunlight of conidia among isolates of entomopathogenic hyphomycetes. *Mycopathologia* **135**, 171–181.

Fernandes, E.K.K., Rangel, D.E., Moraes, A.M., Bittencourt, V.R. and Roberts, D.W. (2007) Variability in tolerance to UV-B radiation among *Beauveria* spp. isolates. *J Invertebr Pathol* **96**, 237–243.

Fernandes, E.K.K., Rangel, D.E., Braga, G.U.L. and Roberts, D.W. (2015) Tolerance of entomopathogenic fungi to ultraviolet radiation: a review on screening of strains and their formulation. *Curr Genet* **61**, 427–440.

Harrington, L.C., Edman, J.D. and Scott, T.W. (2001) Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? *J Med Entomol* **38**, 411–422.

Le Grand, M. and Cliquet, S. (2013) Impact of culture age on conidial germination, desiccation and UV tolerance of entomopathogenic fungi. *Biocontrol Sci Technol* **23**, 847–859.

Leles, R.N., Sousa, N.A., Rocha, L.F.N., Santos, A.H., Garcia Silva, H.H. and Luz, C. (2010) Pathogenicity of some hypocrealean fungi to adult *Aedes aegypti* (Diptera: Culicidae). *Parasitol Res* **107**, 1271–1274.

Lima, W.P., Chiaravalloti Neto, F., Macoris, M.D.L.D.G., Zuccari, D.A.P.C. and Dibo, M.R. (2009) Estabelecimento de metodologia para alimentação de *Aedes aegypti* (Diptera-Culicidae) em camundongos swiss e avaliação da toxicidade e do efeito residual do óleo essencial de *Tagetes minuta L* (Asteraceae) em populações de *Aedes aegypti*. *Rev Soc Bras Med Trop* **42**, 638–641.

Luz, C., Silva, I.G., Cordeiro, C.M.T. and Tigano, M.S. (1999) Sporulation of *Beauveria bassiana* on cadavers of *Triatoma infestans* after infection at different temperatures and doses of inoculum. *J Invertebr Pathol* **73**, 223–225.

Luz, C., Tai, M.H.H., Santos, A.H., Rocha, L.F.N., Albernaz, D.A.S. and Silva, H.H.G. (2007) Ovicidal activity of entomopathogenic hyphomycetes on *Aedes aegypti* (Diptera: Culicidae) under laboratory conditions. *J Med Entomol* **44**, 799–804.

Marcondes, C. and Ximenes, M.F.F.M. (2016) Zika virus in Brazil and the danger of infestation by *Aedes (Stegomyia)* mosquitoes. *Rev Soc Bras Med Trop* **49**, 4–10.

Quaite, F.E., Sutherland, B.M. and Sutherland, J.C. (1992) Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. *Nature* **358**, 576–578.

Rangel, D.E.N., Braga, G.U.L., Flint, S.D., Anderson, A.J. and Roberts, D.W. (2004) Variations in UV-B tolerance and germination speed of *Metarhizium anisopliae* conidia produced on artificial and natural substrates. *J Invertebr Pathol* **87**, 77–83.

Rocha, L.F.N., Inglis, P.W., Humber, R.A., Kipnis, A. and Luz, C. (2013) Ocurrence of *Metarhizium* spp. in Central Brazilian soils. *J Bas Microbiol* **53**, 251–259.

Scholte, E.J., Knols, B.G., Samson, R.A. and Takken, W. (2004) Entomopathogenic fungi for mosquito control: a review. *J Insect Sci* **4**, 19.

Scholte, E.J., Takken, W. and Knols, B.G.J. (2007) Infection of adult *Aedes aegypti* and *Ae. albopictus* mosquitoes with the entomopathogenic fungus *Metarhizium anisopliae*. *Acta Trop* **102**, 151–158.

Snetselaar, J., Andriessen, R., Suer, R.A., Osinga, A.J., Knols, B.G.J. and Farenhorst, M. (2014) Development and evaluation of a novel contamination device that targets multiple life-stages of *Aedes aegypti*. *Parasit Vectors* **7**, 200.

Taylor, B. and Jones, M.D.R. (1969) The circadian rhythm of flight activity in the mosquito *Aedes aegypti* (L.): the phase-setting effects of light-on and light-off. *J Exp Biol* **51**, 59–70.

Throne, J.E., Weaker, D.K., Chew, V. and Baker, J.E. (1995) Probit analysis of correlated data: multiple observations over time at one concentration. *J Econ Entomol* **88**, 1510–1512.

Trpis, M., McClelland, G.A.H., Gillett, J.D., Teesdale, C. and Rao, T.R. (1973) Diel periodicity in the landing of *Aedes aegypti* on man. *Bull WHO* **48**, 623–629.

Vega-Rúa, A., Lourenço de Oliveira, R., Mousson, L., Vazeille, M., Fuchs, S., Yébakima, A., Gustave, J., Girod, R. *et al.* (2015) Chikungunya virus transmission potential by local *Aedes* mosquitoes in the Americas and Europe. *PLoS Negl Trop Dis* **9**, e0003780.

Winston, P.W. and Bates, D.H. (1960) Saturated solutions for the control of humidity in biological research. *Ecology* **41**, 232–237.

Wong, J., Astete, H., Morrison, A.C. and Scott, T.W. (2011) Sampling considerations for designing *Aedes aegypti* (Diptera: Culicidae) oviposition studies in Iquitos, Peru: substrate preference, diurnal periodicity, and gonotrophic cycle length. *J Med Entomol* **48**, 45–52.